

Effect of Mushroom on the Lipid Profile, Lipid Peroxidation and Liver Functions of Aging Swiss Albino Rats

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Abstract: *Introduction:* Mushrooms are a manifestation of a common saying, 'Medicines and foods have a common origin', in constituting both a nutritionally functional food and a source of physiologically beneficial medicine. The present studies were undertaken to investigate the effects of dried mushroom and mushroom extract on the lipid profile, lipid peroxidation and liver function of aging Swiss albino rats as compared with L-carnitine.

Materials & Methods: Male Swiss albino rats of Wister strain of the age of 45 days weighing approximately 320 ± 8 g were used. The animals were fed a basal diet for one week as an adaptation period. The basal diet was formulated including essential ingredients. Water was available *ad libitum*. The animals were divided to five groups. The first group (control) fed the basal diet. The second and the third groups fed L-carnitine (400 and 800 mg/kg body weight/day, respectively) in 0.9% saline at physiological pH. The fifth and sixth groups were fed 15% dried mushroom and 450 mg/kg body weight/day mushroom extract, respectively. Blood samples were taken at the beginning of the experiment and at the end of experiment (4 weeks) from orbital plexus versus by means of fine capillary glass tubes, sera were separated, and lipid profile, lipid peroxidation stage and liver function profile were determined according to the well established methodology after slight modifications.

Results & Conclusion: Mushroom and their extracts were comparable to L-carnitine in controlling lipid peroxidation. Dried mushroom and their extract can improve the antioxidant status during aging and minimize the occurrence of age-associated disorders as a consequence of the involvement of free radicals. The results obtained from the present studies provide new insights into work to be carried out on histological examination of liver tissues of rats fed basal diet, and may project the congestion of the central vein and infiltration with chronic inflammatory cells. These examinations may also show slight hydropic degeneration of hepatocytes and vacuolations of some hepatocytes and small focal hepatic necrosis.

Keywords: L-carnitine, Lipid peroxidation, Lipid profile, Liver function, Mushroom, Mushroom extract, Swiss Albino rat,

INTRODUCTION

In fact, aging is associated with biochemical and structural alterations which are thought to result in motor and cognitive impairments and in increased susceptibility to neurodegenerative diseases [1-4]. The free radical theory of aging proposed that aging is due to the accumulation of unrepaired damage from free radical attack on cellular components. Modern approaches propose that aging is caused by a shift in the balance between the pro-oxidative and anti-oxidative processes in the direction of the pro-oxidative state [1, 2, 5-7]. L-carnitine, a nutrient normally synthesized from methionine and lysine in the liver and kidney. L-carnitine transports long-chain fatty acids (LCFA) across the mitochondrial membrane where they undergo beta-oxidation to produce energy. Carnitine deficiency decreases LCFA availability for oxidation, thereby resulting in LCFA accumulation

in the cytosol, and decreased ketone and energy production. Other L-carnitine functions include the maintenance of adequate free coenzyme-A required for various metabolic pathways, the protection of cells against toxic accumulation of acyl-coenzyme-A compounds by shuttling acyl groups out of the mitochondria, and the storage and transport of energy [8]. Also, L-carnitine supports the immune system and enhances the antioxidant system [9].

Mushrooms are a manifestation of a common saying, 'Medicines and foods have a common origin', in constituting both a nutritionally functional food and a source of physiologically beneficial medicine. Many centuries ago, medicinal properties of mushrooms have been recognized in China, Korea and Japan. Although from ancient times, mushrooms have been treated as a special kind of nutraceutical, they have received a remarkable interest in recent decades. Major medicinal properties attributed to mushrooms include anti-cancer activity, antibiotic activity, antiviral activity, immune response-stimulating effects, anti-hypersensitive and blood lipid lowering effects [10-12]. Mushroom is known to have high amounts of proteins, carbohydrates and fibers and low fat contents [13]. Furthermore, mushroom had significant

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levels of vitamins, namely thiamine, riboflavin, ascorbic acid and vitamin D₂, as well as minerals [14]. Mushroom species had been shown to possess antioxidant capacity in *in-vitro* systems [15-18]. The mushroom *Pleurotus species* (*P. ostreatus*, *P. sajor-caju*, *P. florida*) were reported to have hypocholesterolemic activity in experimental rats [18-20]. It has been reported that the L-carnitine concentration in mushroom ranged from 130 to 533 mg/kg dried mushroom [21]. The free L-carnitine concentration in mushroom ranged from 75 to 385 mg/kg dried mushroom, which represented 70 ± 10% of total carnitine content. Therefore, the present studies were undertaken to investigate the effects of dried mushroom and mushroom extract on the lipid profile, lipid peroxidation and liver function of aging Swiss albino rats as compared with L-carnitine.

MATERIALS AND METHODS

L-carnitine was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Total cholesterol, HDL-cholesterol, LDL-cholesterol, total lipids, alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT), glutathione peroxidase (GSH) and malonaldehyde (MDA) kits were obtained from Randox Laboratories Ltd, England.

Pleurotus ostreatus was cultivated adopting the ‘‘layer spawning’’ method. Whole mushrooms were dried in the shade and then finely powdered. Dried mushrooms were ground to pass through a 60 mesh sieve. Seventy five grams of dried mushrooms were extracted with 225 ml of 95% ethanol using a Soxhlet apparatus. The residue was filtered and concentrated to a dry mass by vacuum distillation and used as mushroom extract.

Male Swiss albino rats of Wister strain of the age of 45 days weighing approximately 320 ± 8 g were used. A total of forty Swiss albinos male rats were raised in the central animal house facility at CET-IFTM, Moradabad, India. The animals were fed a basal diet for one week as an adaptation period. The basal diet was formulated according to AIN [22] and consisted of casein (12%), corn oil (10%), cellulose (5%), salt mixture (4%), vitamin mixture (1%) and starch (68%). Water was available *ad libitum*. The animals were divided to five groups. The first group (control) fed the basal diet. The second and the third groups fed L-carnitine (400 and 800 mg/kg body weight/day, respectively) in 0.9% saline

at physiological pH. The fifth and sixth groups were fed 15% dried mushroom and 450 mg/kg body weight/day mushroom extract, respectively. This study was duly approved by Institutional Animal Ethical Committee.

Blood samples were taken at the beginning of the experiment and at the end of experiment (4 weeks) from orbital plexus versus by means of fine capillary glass tubes according to the method described by Schermer [23]. The blood samples were placed in dry and clean centrifuge tubes and allowed to clot for 1–2 h at room temperature. Serum was removed using a Pasteur pipette and centrifuged for 20 min at 1100 x g. The clean supernatant serum was kept frozen until analysis. Body weights of animals were recorded at the start of the experiment and at the end of experiment. As far as sample size determination was concerned, systematic sampling statistical method was employed in the present study [24].

The serum triacylglycerides, low density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol and total lipid were determined according to the methods described herein [25-29]. Alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) enzymes were measured according to the methods described by research groups [30-32]. Glutathione peroxidase enzyme (GSH) and malonaldehyde (MDA) were determined according to the methods described by Hu, and Jentzsch *et al.* [33, 34], respectively.

The data obtained were analyzed using the analysis of variance (ANOVA) to determine differences [35, 36] and Duncan’s Multiple Range Test (DMRT) to separate the means [37].

RESULTS AND DISCUSSION

The effect of 15% dried mushroom, 450 mg mushroom extract and L-carnitine on total lipid, triglyceride and total cholesterol is shown in Table 1. Total lipid content significantly ($P \leq 0.05$) reduced in albino rats supplemented with mushroom and L-carnitine. The reduction in the total lipids ranged from 7.06 to 14.39%. There was no significant ($P > 0.05$) difference in total lipid between rats supplemented with 400 mg L-carnitine and those supplemented with 15% dried mushroom. Albino rats supplemented with 800 mg L-carnitine had a higher ($P \leq 0.05$) total lipid content compared to those supplemented with 450 mg mushroom extract. Diet

Table 1. Effect of Dried Mushroom, Mushroom Extract and L-Carnitine on Lipid Profile of Aging Rats

Groups	Total Lipids (mg/dl)			Triglyceride (mg/dl)			Total Cholesterol (mg/dl)		
	Before	After	% Changes	Before	After	% Changes	Before	After	% Changes
Aged rats control	484.54±8.31	479.03±4.79	-1.14	194.5±8.16	163.3±9.13	-16.07	166.2±4.68	160.7±4.31	-3.29
Aged rats with 400 mg LC	485.21±4.51	445.22±6.96	-8.24	213.5±8.63	146.7±3.64	-31.28	165.49±3.75	139.15 ^b ±4.55	-15.92
Aged rats with 800 mg LC	480.55±5.59	435.70±6.35	-9.33	199.6±8.61	121.5±4.21	-39.13	165.15±3.51	118.16±3.61	-28.45
Aged rats with 15% DM	476.60±8.23	442.95±6.4	-7.06	215.1±5.11	139.5±3.41	-35.15	163.61±4.01	130.45±4.72	-20.27
Aged rats with 400 mg ME	485.02±6.53	415.22±2.18	-14.39	215.9±4.31	121.5±1.39	-43.72	160.52±4.53	120.00±3.65	-25.24

Values are expressed in blood serum samples as means ±SD of 8 rats from each group with triplicates in each set of experiment.

supplemented with mushroom and L-carnitine resulted in a significant ($P \leq 0.05$) decrease in triglyceride and total cholesterol level. Triglyceride was observed to reduce by 31.28–43.72%. However, total cholesterol reduced by 15.92–28.45%. Supplementation with 450 mg mushroom extract and 800 mg L-carnitine were more ($P \leq 0.05$) effective in reducing triglyceride and total cholesterol than those supplemented with 15% dried mushroom and 400 mg L-carnitine. On the other hand, supplementation with 450 mg mushroom extract and 800 mg L-carnitine were similar ($P > 0.05$) in reducing triglyceride and total cholesterol levels. Supplementation with 15% dried mushroom and 400 mg L-carnitine were also similar ($P > 0.05$) in reducing triglyceride and total cholesterol. It has been observed that rats fed a semisynthetic diet containing 0.3% cholesterol and supplemented with 5% dried whole oyster mushroom had reduced serum and liver cholesterol levels by 34 and 58%, respectively [38]. Panchamoorthy and Carani [39] reported that treated rats with L-carnitine caused a significant reduced in TG as compared to untreated rats. L-carnitine is known to promote the transport of cytosolic long-chain fatty acids into the mitochondrial matrix for β -oxidation, thereby providing mitochondrial energy [40, 41]. L-carnitine may lower plasma TG by increasing the utilization and/or oxidation of fatty acids for energy or possibly by altering very low-density lipoprotein synthesis [42].

The data expressed in Table 2 indicate that the high density lipoprotein in rats was not affected ($P > 0.05$) as a consequence of the supplementation with 15% dried mushroom and 400 mg L-carnitine. However, rats supplemented with 450 mg mushroom extract and 800 mg L-carnitine had a higher ($P \leq 0.05$) high density lipoprotein compared to those of the control sets. High density lipoprotein was monitored to enhance in these albino rats by 24.11–30.44%. Low density lipoprotein ($P \leq 0.05$) reduced in albino rats supplemented with mushroom and L-carnitine by 30.36–55.76%. Supplementation of rats with 450 mg mushroom extract and 800 mg L-carnitine were more ($P \leq 0.05$) effective in lowering low density lipoprotein than those supplemented with 15% dried mushroom and 400 mg L-carnitine. On the other hand, supplementation of rats with 450 mg mushroom extract and 800 mg L-carnitine were similar ($P > 0.05$) in decreasing low density lipoprotein. Supplementation of rats with 15% dried mushroom and 400 mg L-carnitine were also similar ($P > 0.05$) in lowering low density lipoprotein. Very

low density lipoprotein in rats was ($P \leq 0.05$) reduced by the supplementation with mushroom and L-carnitine. Very low density lipoprotein was reduced in these rats by 32.33–42–21%. Supplementation of rats with 450 mg mushroom extract and 800 mg L-carnitine were more ($P \leq 0.05$) effective in reducing very low density lipoprotein than those supplemented with 15% dried mushroom and 400 mg L-carnitine. Diet supplemented with 450 mg mushroom extract and 800 mg L-carnitine did not significantly ($P > 0.05$) differ in their effect on very low density lipoprotein. Besides, no significant ($P > 0.05$) difference was observed in very low density lipoprotein between albino rats supplemented with 15% dried mushroom and those supplemented with 400 mg L-carnitine. These results are in agreement with those reported earlier [42, 43] highlighting that L-carnitine well stabilizes the level of lipids peroxidation, decreases concentration of total lipids, triglycerides, total cholesterol, phospholipids, and lipoproteins of low and very low density, in the Swiss albino rats blood sera.

Table 3 indicates that the aspartate amino transferase (AST) enzyme in rats was assessed to significantly ($P \leq 0.05$) reduce by the supplementation of diet with mushroom and L-carnitine. Mushroom reduced AST enzyme by 38.64–41.46%. However, L-carnitine reduced it by 24.58–42.80%. Swiss albino rats supplemented with 400 mg L-carnitine showed a higher ($P \leq 0.05$) AST enzyme compared to those supplemented with mushroom and 800 mg L-carnitine. Diet supplemented with 450 mg mushroom extract and 800 mg L-carnitine were not significantly ($P > 0.05$) differed in their impact on AST enzyme. Further, diet supplemented with mushroom and L-carnitine had a lower ($P \leq 0.05$) alanine amino transferase (ALT) enzyme compared to that of the control sets. Mushroom and L-carnitine reduced ALT enzyme by 36.59–45.61% and 22.40–36.99%, respectively. Diet supplemented with 15% dried mushroom, 450 mg mushroom extract and 800 mg L-carnitine appeared to be more effective ($P > 0.05$) in decreasing ALT enzyme compared to those supplemented with 400 mg L-carnitine. No significant ($P > 0.05$) difference was found in ALT enzyme among rats supplemented with 15% dried mushroom, 450 mg mushroom extract and those supplemented with 800 mg L-carnitine. The alkaline phosphatase (ALP) enzyme in rats was observed to significantly ($P \leq 0.05$) reduce by the supplementation with mushroom and L-carnitine. Mushroom reduced ALP enzyme by 22.19–32.71%. However, L-carnitine re-

Table 2. Effect of Dried Mushroom, Mushroom Extract and L-Carnitine on Lipoprotein of Aging Rats

Groups	High Density Lipoproteins (mg/dl)			Low Density Lipoproteins (mg/dl)			Very Low Density Lipoproteins (mg/dl)		
	Before	After	% Changes	Before	After	% Changes	Before	After	% Changes
Aged rats control	49.18±2.56	53.31±4.07	+8.40	76.58±4.83	72.37±3.03	-5.49	39.52±2.54	33.06±2.63	-16.34
Aged rats with 400 mg LC	48.36±3.14	55.46±5.44	+14.68	71.83±2.51	50.02±0.36	-30.36	41.35±1.83	27.97±0.64	-32.33
Aged rats with 800 mg LC	51.01±3.52	63.31±3.01	+24.11	76.04±4.69	33.64±0.11	-55.76	39.17±2.31	24.31±1.05	-37.93
Aged rats with 15% DM	47.65±2.76	52.73±3.61	+10.66	73.89±4.72	51.65±3.10	-30.09	41.06±1.49	27.09±0.88	-34.02
Aged rats with 400 mg ME	49.25±3.62	64.24±2.61	+30.44	71.45±5.88	33.40±1.18	-53.25	41.77±1.21	24.14±1.41	-42.21

Values are expressed in blood serum samples as means \pm SD of 8 rats from each group with triplicates in each set of experiment.

duced it by 22.14-49.26%. The Diet supplemented with 400 mg L-carnitine had a higher ($P \leq 0.05$) ALP enzyme compared to those supplemented with 800 mg L-carnitine. Diet supplemented with 15% dried mushroom had a higher ($P \leq 0.05$) ALP enzyme compared to those supplemented with 450 mg mushroom extract. The diet supplemented with 15% dried mushroom and 400 mg L-carnitine were not significantly ($P > 0.05$) differed in their impact on ALP enzyme. L-carnitine and mushroom restores the changes of ALT, AST and ALP activities due to their antioxidant effects and their ability to act as a radical scavenger, thereby protecting membrane permeability. Augustyniak and Skrzydlewska (2009) [44] found that ALT and AST after ethanol intoxication their activity increased by about 80%. L-carnitine partly prevented these changes. It was manifested by a statistically significant decrease in the activity of ALT and AST, by about 20% in comparison with the ethanol group.

The Data shown in Table 4 reflect that the MDA ($P \leq 0.05$) got reduced by 11.92-33.79% in albino rats supplemented with diet containing mushroom and L-carnitine. Supplementation with 450 mg mushroom extract and 800 mg L-carnitine were more ($P \leq 0.05$) effective in decreasing MDA compared to those supplemented with 15% dried mushroom and 400 mg L-carnitine. On the other hand, supplementation with 450 mg mushroom extract and 800 mg L-carnitine were similar ($P > 0.05$) in reducing MDA. Supplementation of diets with 450 mg mushroom extract and 400 mg L-carnitine were also similar ($P > 0.05$) in reducing

MDA. Rats supplemented with the diet containing 15% dried mushroom had higher ($P \leq 0.05$) MDA compared to those supplemented with 400 mg L-carnitine. It has earlier been reported that administration of L-carnitine to rats intoxicated with ethanol significantly protects lipids and proteins against oxidative modifications in the serum and liver. The level of MDA was decreased by about 30%, in the blood serum in comparison to the ethanol group [44].

Glutathione peroxidase (GSHPx) is known to perform a key role in co-ordinating the innate antioxidant defense mechanisms. It is involved in the maintenance of the normal structure and function of cells, probably by its redox and detoxification reactions [1, 2, 45]. The GSHPx in rats was monitored to be significantly ($P \leq 0.05$) enhanced by the supplementation with mushroom and L-carnitine. Mushroom increased GSHPx by 58.43-85.50%. However, L-carnitine increased it by 60.15-129.69%. Rats supplemented with 450 mg L-carnitine and 15% dried mushroom had a lower ($P \leq 0.05$) GSHPx compared to those supplemented with 800 mg L-carnitine and 450 mg mushroom extract. Supplementation with 15% dried mushroom and 400 mg L-carnitine were not significantly ($P > 0.05$) differed in their effect on GSHPx. Supplementation of rats with 800 mg L-carnitine was more ($P \leq 0.05$) effective in increasing GSHPx compared to those supplemented with 400 mg L-carnitine, 15% dried mushroom and 450 mg mushroom extract. According to Augustyniak and Skrzydlewska [44] L-carnitine has been reported to cause a significant increase in the liver and blood

Table 3. Effect of Dried Mushroom, Mushroom Extract and L-Carnitine on Liver Functions of Aging Rats

Groups	Aspartate Aminotransferase (AST) (U/ml)			Alanine Aminotransferase (ALT) (U/ml)			Alkaline Phosphatase (ALP) (U/ml)		
	Before	After	% Changes	Before	After	% Changes	Before	After	% Changes
Aged rats control	82.15±2.11	76.35±2.40	-7.06	42.60± 1.73	40.15±3.40	-5.81	62.11± 3.14	62.52±2.41	+0.66
Aged rats with 400 mg LC	80.98±3.52	61.87±3.52	-24.58	44.12± 1.92	34.26±3.30	-22.40	61.64±3.42	48.61±4.91	-21.14
Aged rats with 800 mg LC	81.26±3.41	46.48±1.41	-42.80	42.15±1.79	26.56±1.52	-36.99	62.91±3.52	31.92±2.11	-49.26
Aged rats with 15% DM	82.95±2.35	50.89±2.13	-38.64	42.50±2.31	26.95±1.31	-36.59	64.45±2.14	50.15±2.84	-22.19
Aged rats with 400 mg ME	83.79±2.42	49.05±2.65	-41.46	42.56±1.98	23.15±2.01	-45.61	61.40±2.64	41.32±1.21	-32.71

Values are expressed in blood serum samples as means ±SD of 8 rats from each group with triplicates in each set of experiment.

Table 4. Effect of Dried Mushroom, Mushroom Extract and L-Carnitine on MDA and GSHPx of Aging Rats

Groups	MDA(n mol/ml)			GSHPx (U/ml)		
	Before	After	% Changes	Before	After	% Changes
Aged rats control	24.81±2.11	25.39±1.46	+1.82	11.1±3.50	11.54±2.71	+1.65
Aged rats with 400 mg LC	26.39±2.14	19.04±0.54	-27.9	13.11±3.38	21.96±2.61	+60.15
Aged rats with 800 mg LC	25.92±1.61	17.16±1.31	-33.79	12.04±2.91	31.59±2.91	+129.69
Aged rats with 15% DM	24.58±2.41	21.65±1.51	-11.92	10.98±4.61	19.41±1.61	+58.43
Aged rats with 400 mg ME	24.45±1.46	18.15±0.90	-25.7	14.01±4.01	25.98±2.51	+85.50

Values are expressed in blood serum samples as means ±SD of 4 rats from each group with triplicates in each set of experiment.

Table 5. Effect of Dried Mushroom, Mushroom Extract and L-Carnitine on Food Intake and Body Weight of Aging Rats

Groups	Food Intake (g/day)	Body Weight (g)	
		Initial	Final
Aged rats control (G1)	9.4 ± 1.15	316.6 ± 9.85	328.5 ± 8.51
Aged rats with 400 mg LC (G2)	17.6 ± 2.14	318.2 ± 8.38	276.6 ± 8.15
Aged rats with 800 mg LC (G3)	18.3 ± 1.99	311.6 ± 9.16	269.6 ± 7.63
Aged rats with 15% DM (G4)	17.4 ± 1.86	313.4 ± 9.47	318.4 ± 8.05
Aged rats with 400 mg ME (G5)	17.7 ± 1.91	315.8 ± 8.54	319.57 ± 8.17

Values are means ±SD of 8 rats from each group with triplicates in each set of experiment.

serum GSH level by more than 20%. An increase in the levels of GSHPx in aged rats treated with mushroom extract as a source of antioxidant has also been recently reported [45, 46].

Table 5 highlights the effect of dried mushroom, mushroom extract and L-carnitine on food intake and body weight of Swiss albino rats. Either L-carnitine or mushroom significantly ($P \leq 0.05$) increased food intake and reduced body weight in rats. There was no significant ($P > 0.05$) variation in food intake between rats supplemented with L-carnitine and mushroom. Supplementation of rats with L-carnitine was more ($P \leq 0.05$) effective in reducing body weight than those supplemented with mushroom. Supplemented rats with 400 mg L-carnitine and 800 mg L-carnitine were not significantly ($P > 0.05$) distinct in their effect on body weight. Similar effect was monitored in rats supplemented with 15% dried mushroom and 450 mg mushroom extract. The rationale for L-carnitine supplementation as a weight-loss agent is based on the assumption that regular oral ingestion of the substance increases its intracellular concentration. This would trigger increased fat oxidation and gradual reduction of the body's fat reserves [47, 48].

CONCLUDING REMARKS

Conclusively, mushroom and their extracts were comparable to L-carnitine in controlling lipid peroxidation. Dried mushroom and their extract can improve the antioxidant status during aging and minimize the occurrence of age-associated disorders as a consequence of the involvement of free radicals. The results obtained so far from the present studies provide new insights into work to be carried out on histological examination of liver tissues of rats fed basal diet, and may project the congestion of the central vein and infiltration with chronic inflammatory cells. These examinations may also show slight hydropic degeneration of hepatocytes and vacuolations of some hepatocytes and small focal hepatic necrosis.

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